The Association between HOXB7 Expression in Prostate Adenocarcinoma Tissues and Poor Prognosis of Patients

Susu Tang[1], Lirong Xiao[2,*]
[1]The Affiliated Nanhua Hospital, Department of Pain Rehabilitation, Hengyang Medical School, University of South China, Hengyang 421002, Hunan, China
[2]The Affiliated Nanhua Hospital, Department of Spine Surgery, Hengyang Medical School, University of South China, Hengyang 421002, Hunan, China
**Corresponding Author.*

Abstract: We accessed the TCGA database to analyze the HOXB7 mRNA expression in adenocarcinoma tissues of the prostate and explore its relationship with the Gleason Score, molecular characteristics of tumor tissues, patient's lymph node metastasis status, age, race, and survival period. Meanwhile, immunohistochemistry was utilized to detect the HOXB7 expression in 10 samples of prostate adenocarcinoma tissues and nearby normal tissues. The prostate cancer tissues were classified according to the Gleason score. The analysis revealed that HOXB7 mRNA expression within the tissues of prostate adenocarcinoma was linked to the molecular characteristics of prostate adenocarcinoma tissues and the lymph node metastatic status of patients, but was independent of patient age and race. Immunohistochemistry results demonstrated that there were statistically meaningful differences in the average optical density values of HOXB7 staining between prostate adenocarcinoma tissues and adjacent tissues ($t = 18.442$, $P < 0.001$). Patients with high HOXB 7 expressions in prostate cancer tissue tended to have a poor prognosis ($p = 0.0036$).

Keywords: HOXB7; Brostate Adenocarcinoma; Differential Expression; Prognosis; Correlation Analysis

1. Introduction

Homeobox7 (HOXB7) is a gene encoding HOXB7 protein in HOXB cluster, located on chromosome 17. As a transcription regulator, HOXB7 plays a key role in DNA synthesis and transcription, and its abnormal expression can lead to different types of malignant tumors [1]. Existing studies have shown that overexpression of HOXB7 has the ability to facilitate the proliferation and metastasis of esophageal cancer [2], gastric cancer [3], and colorectal cancer [4]. In addition, HOXB7 plays a crucial role in the drug resistance of stomach cancer, esophageal carcinoma [1] and ovarian cancer cells [2]. Cheng demonstrated that reducing the HOXB7 expression can enhance the sensitivity of esophageal cancer cells [1] and cervical cancer cells [1] to radiotherapy.

At present, the expression of HOXB 7 and its clinical significance in prostate adenocarcinoma are not completely clear. This article intends to analyze the expression of HOXB 7 through bioinformatics methods and immunohistochemistry, and investigate its clinical significance, to offer new targets for the prevention and management of prostate cancer.

2. Methods

2.1 Experimental Methods

2.1.1 Analyzing the expression differences of HOXB7 mRNA between prostate adenocarcinoma and normal prostate tissues from the TCGA database

Prior to analysis, samples with missing clinical data were excluded. Subsequently, data for normal prostate samples and prostate adenocarcinoma samples were downloaded through the TCGA data repository. These data were then employed to analyze the expression with differential characteristics of HOXB7 mRNA between prostate adenocarcinoma and normal prostate tissues.

2.1.2 Analysis of the influencing factors of HOXB7 mRNA expression in prostate adenocarcinoma tissues

We retrieved data from the TCGA data bank to investigate the correlation between HOXB7 mRNA expression and Gleason score, to analyze the relationships of HOXB 7 expression in prostate adenocarcinoma tissues with Gleason Score and molecular characteristics of tumor tissues, lymph node metastasis status of patients, age and race of patients.

2.1.3 Immunohistochemical detection of prostate adenocarcinoma tissues and Gleason score classification

To validate the findings from the TCGA database analysis, we employed immunohistochemistry to assess the expression of HOXB7 in 10 cases of prostate adenocarcinoma tissues and their adjacent non cancerous tissues. Meanwhile, the Gleason score classification was carried out on the prostate cancer tissues. HOXB 7 was purchased from Abnova Corporation (Abnova), and the second antibody was purchased from Jiangsu KeyGEN BioTECH Co., Ltd. At 10×20 times field of vision, and the staining results were examined by optical density using Image-Pro Plus image analysis software. Statistical analysis was carried out with the utilization of SPSS19.0 software. Measurement data were presented in the form of mean ± standard deviation ($\bar{x} \pm s$), and a P - value less than 0.01 was regarded as statistically significant.

2.1.4 Leveraging the TCGA database, we delved into the correlation between the expression quantities of HOXB7 mRNA in prostate gland adenocarcinoma tissues and the overall survival (OS) of patients.

The Cancer Genome Atlas database was harnessed to explore the relationship between the expression amounts of HOXB7 mRNA in prostatic adenocarcinoma tissues and how long the patients survived.

2.2 Statistical Analysis

R (version x64 3.5.1) was used to perform all statistical analyses. Wilcoxon test was applied to assess the levels of HOXB7 mRNA expression in prostate cancer tissues and normal prostate tissues. Kaplan-Meier analysis was performed to compare the survival durations between the HOXB7 mRNA high-expression group and the low-expression group. The log rank test was used to calculate the P value. $P<0.05$ indicates that it is statistically significant.

3. Results

3.1 The HOXB7 mRNA Expression in Pan-Cancer Tissues is Compared to that in Their Matched Normal Tissues

Figure 1 graphically illustrates the expression levels of HOXB7 mRNA in pan - cancer tissues and their matched normal tissues. As depicted in Figure 1A, it is evident that HOXB7 expression is upregulated in the overwhelming majority of cancers. Furthermore, Figure 1B convincingly demonstrates that the HOXB7 mRNA expression is markedly higher in pan - cancer tissues compared with the matched normal tissues, highlighting a potentially significant biomarker - related difference between cancerous and normal states.

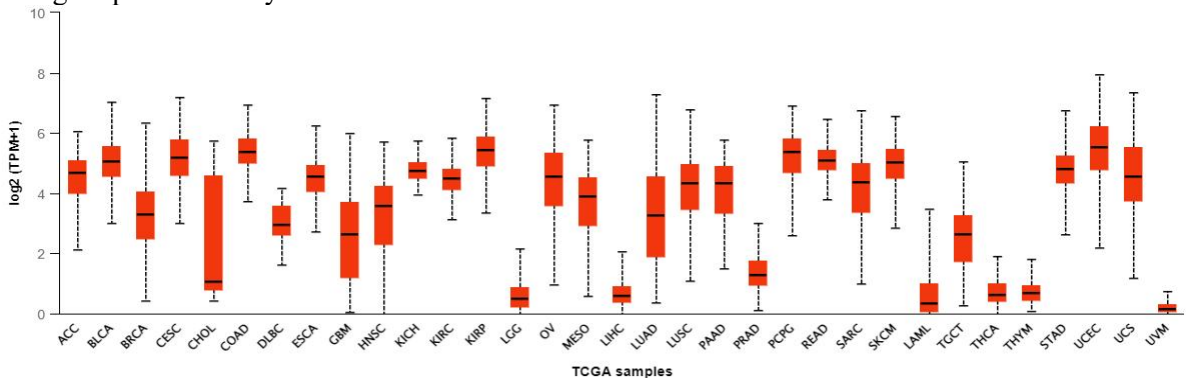
3.2 The HOXB7 mRNA Expression in Adenocarcinomatous Tissues of the Prostate Gland and Factors Affecting Its Expression

After eliminating samples with missing clinical details, we downloaded 52 prostate normal tissues samples and 497 prostate adenocarcinoma tissues samples from the TCGA database. The HOXB7 mRNA expression is significantly lower in prostate adenocarcinoma - associated tissues than in normal prostate tissues ($p = 8.86 \times 10^{-3}$) (Figure 2a). Further analysis revealed that the HOXB7 mRNA expression in the tissues of prostate adenocarcinoma was correlated with the Gleason score. Specifically, the higher the Gleason score, the higher the expression of HOXB7 mRNA (Figure 2b). The lower expression of HOXB7 mRNA in 497 prostate adenocarcinoma samples from the TCGA database may be related to the low Gleason score (Table 1). The analysis further indicated that the HOXB7 mRNA expression level in adenocarcinoma tissues of the prostate gland was associated with the lymph node metastatic status of patients (Figure 2c) as well as the molecular characteristics of prostate adenocarcinoma tissues (Figure 2d). Notably, this correlation was independent of patient age and race (Figure 2e, f).

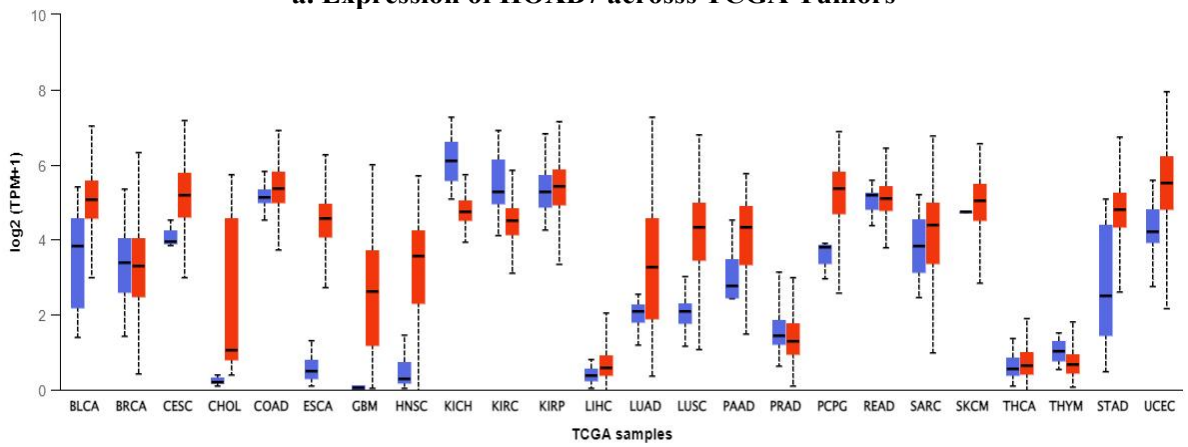
3.3 The Immunohistochemistry Resultes of HOXB 7 Expression in Prostate Adenocarcinoma Tissues and

Paracancerous Tissues.

Immunohistochemistry showed that HOXB 7 staining was brownish yellow or tan, with the main expression sites in the cytoplasm and cell membrane. The in the paracancerous tissues were no or less colored (Figure 3a); The prostate adenocarcinoma tissues had more particles of brownish yellow or tan in the cytoplasm and cell membrane (Figure 3b). The average optical density values of HOXB 7 staining in prostate adenocarcinoma tissues and paracancerous tissues were 0.375 ± 0.019 and 0.095 ± 0.027 individually, having statistically significant distinctions (t=18.442, P <0.001) (Figure 3c). The prostate adenocarcinoma tissues of 10 cases were classified by Gleason score classification, including 6 Gleason score 9 (60%), 3 Gleason score 9 (30%) and 1 Gleason score 6 (10%).

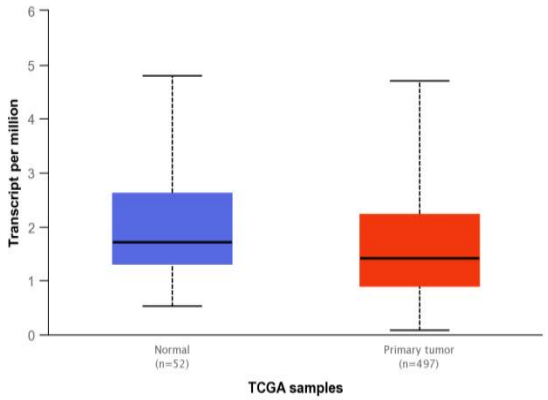


a. Expression of HOXB7 acrosss TCGA Tumors

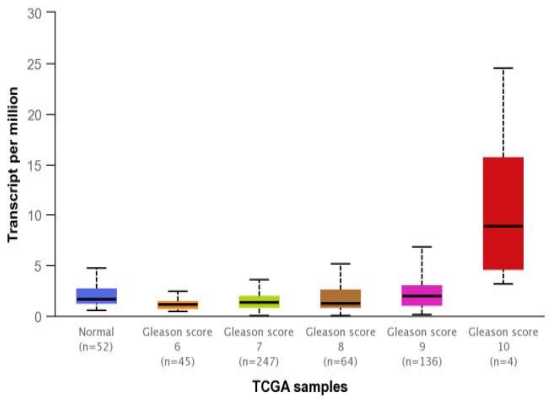


b. Expression of HOXB7 acrosss TCGA cancer with Tumor and Tormal Samples. Blue: Normal; Red: Tumor

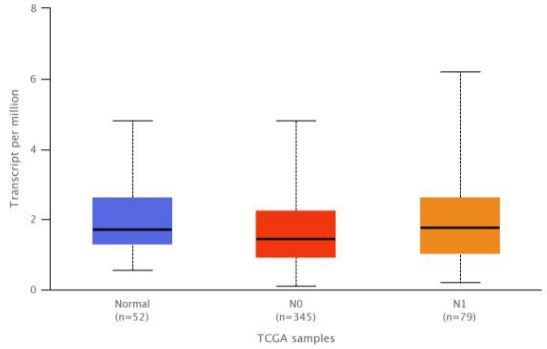
Figure 1. Expression of HOXB7 mRNA in Pan Cancerous Tissues



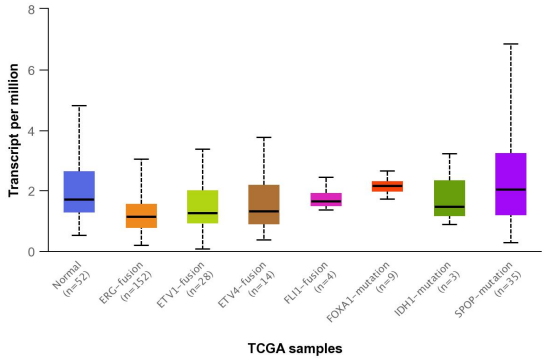
a. Expression difference of HOXB7 between Prostate Adenocarcinoma Tissues and Normal Prostate Tissues.



b. The Correlation between the Expression Level of HOXB7 and the Gleason Score in Prostate Adenocarcinoma Tissues.



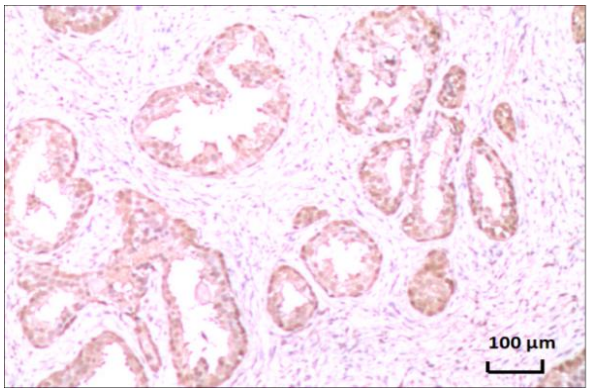
c. Relationship between HOXB7 Expression Level and Lymph Node Metastasis Status in Prostate Adenocarcinoma Tissues



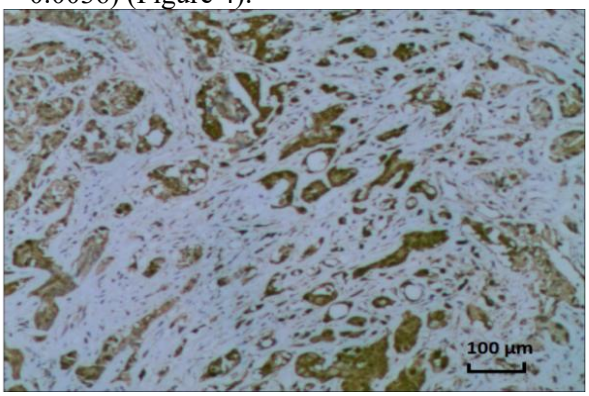
d. The Association of HOXB7 mRNA Expression with the Molecular Features of Prostate Adenocarcinoma Tissues

Table 1. Gleason Score Classification of 497 Prostate Adenocarcinoma Tissues Samples in the TCGA Database

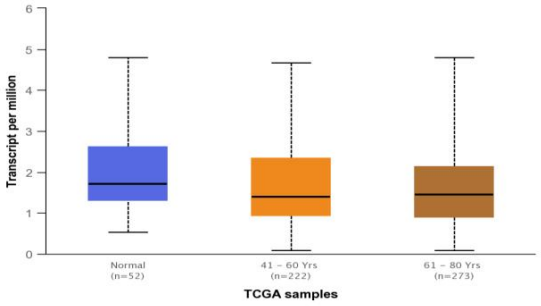
Gleason score	Classification	n (%)
	Gleason score 6	45 (9.0%)
	Gleason score 7	247 (49.8%)
	Gleason score 8	64 (12.9%)
	Gleason score 9	136 (27.4%)
	Gleason score 10	4 (0.9%)



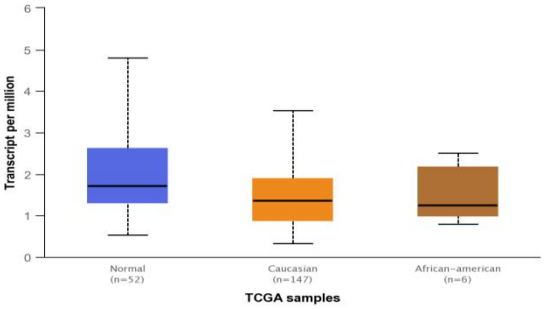
a. The Expression of HOXB 7 in the Paracancerous Tissues



b. The Expression of HOXB 7 in the Prostate Adenocarcinoma Tissues



e. The Correlation between HOXB7 Expression Quantity and the Age of Prostate Adenocarcinoma Patients

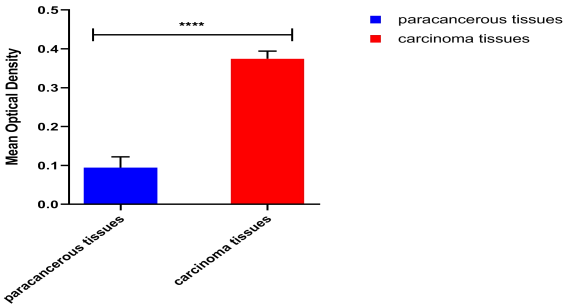


f. The Association between the HOXB7 Expression Abundance and the Race of Patients with Prostate Adenocarcinoma

Figure 2. The HOXB7 mRNA Expression in Adenocarcinoma Tissues of the Prostate and Its Influence Factors

3.4 The HOXB7 mRNA Expression is Associated with the Survival Duration of Individuals Suffering from Prostate Adenocarcinoma.

Analysis of the TCGA database revealed a significant association between the HOXB7 mRNA expression in prostate adenocarcinoma tissue and how long patients survived (p = 0.0036) (Figure 4).



c. The Histogram of HOXB 7 Expression in Prostate Adenocarcinoma Tissues and Paracancerous Tissues

Figure 3. The Expression of HOXB 7 in Prostate Adenocarcinoma Tissues and Paracancerous Tissues(10×20)

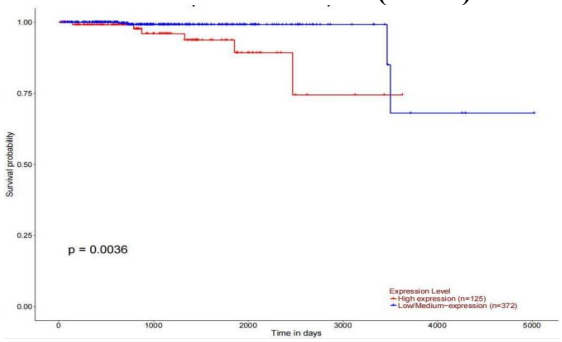


Figure 4. Effect of HOXB7 Expression Level on Prostate Adenocarcinoma Patient Survival

4. Discussion

Prostate adenocarcinoma is a common tumor among elderly men. In 2020, there were 10.7 million newly - diagnosed cancer cases among men across the globe, of which 1.41 million were prostate adenocarcinoma, ranking second in the incidence of male malignant tumors [1]. Early and localized prostate adenocarcinoma can be cured by surgery or radical radiotherapy, but for locally advanced prostate adenocarcinoma and metastatic prostate adenocarcinoma, comprehensive treatment focusing on endocrine therapy [2] generally adopted. However, after 18-24 months of treatment, most patients progress to hormone resistant prostate adenocarcinoma [3], and follow-up treatment methods are limited and the effect is not very satisfactory, it is urgent to further improve the research on its mechanism and explore more effective means of diagnosis and treatment.

We downloaded the samples of 52 normal prostate tissues and 497 prostate adenocarcinoma tissues from TCGA database, and the HOXB7 mRNA expression in prostatic carcinoma tissues was lower compared with that in normal prostate tissues. But, our immunohistochemical findings indicated that HOXB7 mRNA expression was elevated in prostate cancer tissues compared to adjacent non-cancerous tissues. Our results are consistent with those reported by DENG Zhi-hai et al. [5], but seem contradictory with the TCGA database results. In fact, many factors affect the expression of HOXB 7 in prostate cancer tissues, including tumor tissue Gleason score, molecular characteristics of tumor tissue, and lymph node metastasis status of patients, etc. Among them, the Gleason score in tumor tissue was higher, HOXB 7 expression was also even higher in prostate cancer tissues [6]. Our analysis found that more than half of 497cases prostate adenocarcinoma tissues samples from the TCGA database were between Gleason score 6 and Gleason score 7, and lower expression of HOXB7 mRNA may be related to the low Gleason score. While we collected 10 cases tissues samples of prostate adenocarcinoma, Gleason score was higher, among which including 6 cases Gleason score 9 (60%), 3 cases Gleason score 9 (30%) and 1 case Gleason score 6 (10%), and higher expression of HOXB7 mRNA may be related to the higher Gleason score [7,8].

There are few reports on the clinical significance of HOXB 7 expression in prostate adenocarcinoma, and a few articles suggest that the high expression of HOXB 7 in prostate adenocarcinoma may be related to the malignancy and disease progression of prostate adenocarcinoma. Hong and Shi et al. [7,8] discovered that enhancing the expression of HOXB7 can promote the growth and motility of prostate adenocarcinoma cells. We utilized the UALCAN website to gain access to the TCGA database, examined the correlation between HOXB7 mRNA expression levels in prostate adenocarcinoma tissues and patient survival duration, our analysis found that prostate adenocarcinoma patients with elevated HOXB7 expression have a worse prognosis. In summary, HOXB7 expression in prostate adenocarcinoma tissues is linked to the Gleason score, the molecular characteristics of tumor tissues and the lymph node metastatic status of patients.

References

[1] Zhong Y, Zhang Y, Ma D, et al. Inhibition of HOXB7 suppresses p27-mediated acute lymphoblastic leukemia by regulating basic fibroblast growth factor and ERK1/2. Life

Sci. 2019 Feb 1;218:1-7.

[2] Cheng W, Shi X, Lin M, et al. LncRNA MAGI2-AS3 overexpression sensitizes esophageal cancer cells to irradiation through down-regulation of HOXB7 via EZH2. Front Cell Dev Biol. 2020 Nov 24;8:552822.

[3] Yuan C, Xie Y, Sheng X, et al. Role of HOXB7 in promoting gastric cancer progression and oxaliplatin (L-OHP) resistance. Int J Clin Exp Pathol. 2020 Jun 1;13(6):1381-1389. eCollection 2020

[4] Feng W, Gong H, Wang Y, et al. circIFT80 functions as a ceRNA of miR-1236-3p to promote colorectal cancer progression. Mol Ther Nucleic Acids. 2019 Dec 6; 18:375-387.

[5] Deng Zhi-hai, Feng Zhen-hua, Huang Qiang, Deng Jian-zhong. Expression and clinical significance of HOXB7 in prostate cancer. JOURNAL OF GUANGDONG MED, 2017, 35(05):493-495.

[6] Song Z, Liao Z, Cui Y, Yang C. The relationship between homeobox B7 expression and the clinical characteristics of patient with prostate cancer. J Cell Biochem. 2019 Apr; 120(4):6395-6401.

[7] Hong Z, Fu W, Wang Q, et al. MicroRNA-384 is lowly expressed in human prostate cancer cells and has anti-tumor functions by acting on HOXB7. Biomed Pharmacother. 2019 Jun;114:108822.

[8] Shi Z, Zhang H, Jie S, et al. Long non-coding RNA SNHG8 promotes prostate cancer progression through repressing miR-384 and up-regulating HOXB7. J Gene Med. 2021 Mar; 23(3):e3309.

